

Animals: Timed pregnant Sprague-Dawley rats were obtained on gestation day 3 and randomly assigned to treatment groups so that similar mean body weights were achieved at the start of the study. The first 20 females per dose group to litter were used for the study.

Diet: certified rodent diet and tap water purified via reverse osmosis were provided *ad libitum*.

Drug Dose and Route of Administration: CP-99,219-27 (trovafloxacin mesylate) was suspended in 0.5% aqueous methyl cellulose and administered via oral gavage once daily from day 6 of gestation to lactation day 21 at a dose volume of 10 ml/kg. Treatment groups were as follows:

1. Vehicle control
2. 5 mg/kg
3. 15 mg/kg
4. 75 mg/kg

Length of Study: Presumed pregnant females were treated from gestation day 6 to lactation day 21 (as applicable). In order for a litter to continue in the study, on lactation day 4 it had to contain at least 7 pups, with at least 2 pups of each sex. Those not meeting these criteria were sacrificed. Litters containing more than 8 live pups were culled to 8 on lactation day 4 by randomly selecting 4 males and 4 females. Dams completing the study were sacrificed on lactation day 21. At this time, 2 pups per sex per litter was randomly chosen for more behavioral and developmental testing. Of these pups, one male and one female from each litter also underwent reproductive testing.

Results: One dam in the 75 mg/kg group died on gestational day 24 as a result of dystocia. No other dams died during the study, although dystocia was observed in 4 other dams in the 75 mg/kg group. Postpartal pup mortality (deaths during lactation days 1-4) was high in these litters, and none met the minimum criteria for numbers of pups, so they did not continue on study after day 4 of lactation. Salivation was observed sporadically in one dam from the 75 mg/kg group during the treatment period.

No treatment-related effects on body weight gain were observed in the dams during gestation or lactation. Additionally, no effects on food consumption were observed. Necropsy did not reveal any gross changes in the F0 dams attributable to trovafloxacin.

The number of implantation sites was similar among treatment groups, as was the number of pups born per litter. Compared to control, the number pups born alive was reduced in the 75 mg/kg dose group (12.8 ± 2.2 vs. 12.3 ± 2.3) with a concomitant increase in the number born dead (0.1 ± 0.2 vs. 1.5 ± 1.5). The mean length of gestation was significantly ($p \leq 0.05$) increased in the treated dams (5 mg/kg: 21.45 ± 0.60 d, 15 mg/kg: 21.85 ± 0.41 d, 75 mg/kg: 21.94 ± 0.54 d) compared to control (20.95 ± 0.51).

Although an increase in early mortality was apparent in the 75 mg/kg group, the number of pups surviving until lactation day 21 was not different from control in the dams from this group that met the criteria for minimum numbers of pups and continued in the study after culling of the litters to 8 pups on day 4. Mean body weights of the female pups from the

75 mg/kg group were slightly, but significantly ($p < 0.05$) less than control from days 4-14 of lactation; the investigators believed that the effect was likely treatment-related. Trend analysis did not reveal any significant differences in the results of the preweaning developmental indices righting reflex (on a surface or in the air) and visual cliff avoidance. Eye opening occurred earlier than control in the 15 and 75 mg/kg male and female pups and eruption of incisors occurred earlier in female pups from these dose groups. In each case, however, the difference between treated and control animals was less than one day. Trend analysis did not reveal any significant differences between treatment groups for data collected on two pups per sex per litter on postnatal day 21 as part of a "Functional Observation Battery" with the exception of a slightly increased reaction to tail pinch in 75 mg/kg females. The "Functional Observation Battery" for the pups included ease of removal from cage, reaction to handling, rearing (in open field), arousal (level of unprovoked activity in open field), defecation frequency (in open field), urination frequency (in open field), approach response (reaction to placing blunt object approximately 3 cm away from animal's face after open field observation), touch response (approach animal from side and touch rump gently with blunt object), click response (use clicker to make sudden sound approximately 5 cm above the back of the animal), tail pinch response, corneal reflexes, reaction of pupil to light, extensor thrust (animal in supine position pushes with its hind feet in response to pressure from observer's hand), and body temperature. The time to vaginal opening did not differ among the treatment groups, but the time to preputial separation was reduced by about 1.2 days in the 75 mg/kg group. The reductions of time to preputial separation and those observed for eye opening and tooth eruption mentioned above may be related to the delay in parturition, making these animals developmentally older at birth. All pups passed an auditory function test (startle or Preyer response to Galton whistle) administered on postnatal day 30. Examination of pups with an ophthalmoscope during postnatal days 21-28 revealed no evidence of treatment-related anomaly. Motor activity (monitored using a video-based system) on postnatal day 23 did not differ between the treatment groups. Learning and memory were tested using a passive avoidance chamber or the Cincinnati Water Maze (1 pup per sex for each of these tests). Passive avoidance testing (latency period before animals cross into a darkened chamber to avoid an unpleasant stimulus) on postnatal days 63-70 revealed no significantly different trends between the groups. Trials in the Cincinnati Water Maze conducted on postnatal days 55-65 showed no significant differences between the treatment groups for the mean number of errors (either total or per individual trial) when either Path A or Path B was used. There was no significant difference in trial latency for the males, but there was a statistically significant increase in the latency for females of the 75 mg/kg group in the last 2 trials using Path B (more complex than Path A). In the absence of an increased number of errors and because the total latency time for the combined trials for each path did not differ between the control and 75 mg/kg female pups, the increased latency period for the last 2 Path B trials in the high dose female pups does not appear to be toxicologically significant.

The mating index was similar among treatment groups, but the percentage of females that were pregnant was less in the 75 mg/kg group than control. It appears unlikely that the reduced pregnancy rate was really drug-related as a similar study with intravenously administered alatrofloxacin showed no evidence of impairment of fertility in F1 offspring at 50 mg/kg. There appeared to be no differences between control and drug treated F1 rats for parameters such as length of gestation, number of implantation sites, and numbers of live and

dead pups born. Mean pup weights did not differ between litters of control or drug-treated F1 dams. The litter size was similar between groups as were the percentages of viable offspring on postnatal day 4 and at weaning time. No drug-related external or visceral malformations were observed in the F2 pups during a gross necropsy (includes stillborn pups, those culled on day 4, any that died during the study, and those who survived until lactation day 21). Mean body weight of the F1 dams were similar among all groups during gestation and lactation.

Oral dosing with trovafloxacin from gestation day 6 until lactation day 21 was not overtly toxic to pregnant rats with the exception of an increase in mean gestational length at doses ≥ 5 mg/kg. Dystocia was observed in several dams at 75 mg/kg. Early postnatal viability was reduced in the 75 mg/kg group compared to control, most likely due to dystocia. Signs of development such as obtaining the righting reflex and visual cliff avoidance did not differ between control and drug-treated litters, but reductions of time to preputial separation, eye opening and tooth eruption observed in some treated animals in the 15 and/or 75 mg/kg groups may be related to the delay in parturition, making these animals developmentally older at birth. Functional neurological development and learning ability of the F1 pups did not appear to be greatly affected by treatment with trovafloxacin. Sexual development and reproductive capacity of the F1 offspring did not appear to have been affected by trovafloxacin treatment of the F0 dams. The F2 offspring were grossly unaffected by F0 drug treatment. In this study, the NOAEL of trovafloxacin for the dams was less than 5 mg/kg (based upon the increased length of gestation observed in all drug-treated groups), the NOAEL for the F1 offspring was 15 mg/kg (based upon the increased early postnatal mortality observed at 75 mg/kg) and the NOAEL for the F2 offspring was 75 mg/kg.

PHARMACOKINETIC STUDIES: (Contained in Volumes 31-32 of NDA; doses were based upon free trovafloxacin equivalents. Concentrations of trovafloxacin and alatrofloxacin in biological fluids were measured. Concentrations of alatrofloxacin were not always determined in the studies due to its rapid hydrolysis to trovafloxacin.)

Absorption/Pharmacokinetics:

Pharmacokinetics of Trovafloxacin in Sprague-Dawley Rats Following Single Intravenous Administration of Alatrofloxacin or Trovafloxacin and Single Oral Administration of Trovafloxacin (93-099219-4)

Four Sprague-Dawley rats per sex received an IV (intrafemoral vein) dose of 10 mg/kg trovafloxacin mesylate in 1:1 glycerol formal/water and another 4/sex received an oral dose of 50 mg/kg trovafloxacin in 0.5% methylcellulose. Sequential blood samples were obtained 0.08 - 8.0 hours after intravenous dosing and 0.16 - 8.0 hours after oral dosing. For the 10 mg/kg IV dose, the mean clearance of trovafloxacin was 13.7 ± 2.7 ml/min/kg, the volume of distribution was 0.9 ± 0.2 l/kg, the AUC was 12.6 ± 2.8 $\mu\text{g}\cdot\text{hr}/\text{ml}$ and the half life was calculated to be 0.7 h. For the 50 mg/kg oral dose, C_{max} was 11.5 ± 3.3 $\mu\text{g}/\text{ml}$, T_{max} was 0.6 ± 0.3 h, AUC was 42.5 ± 11.2 $\mu\text{g}\cdot\text{hr}/\text{ml}$, and the calculated half life and absolute bioavailability 1.9 h and 67%, respectively. The longer half life after oral dosing is believed to be due to prolonged absorption of trovafloxacin because it has a low aqueous solubility.

Adult vs. neonatal rat pharmacokinetics of trovafloxacin were determined after intravenous doses of 10 or 20 mg/kg alatrofloxacin mesylate in mannitol to 4 adults/sex sampled sequentially (0.08 - 4.0 hours after dosing) or 20 mg/kg to 2 neonatal rats/sex per time point. Comparisons between the pharmacokinetic parameters for the 2 adult dose groups and between adults and neonates were as follows:

	Adult 10	Adult 20	Neonate 20
Clearance (ml/min/kg)	21.0 \pm 0.8	17.9 \pm 0.5	10.5
Volume of Distribution (l/kg)	1.0 \pm 0.1	0.9 \pm 0.1	1.0
AUC (μ g·hr/ml)	8.0 \pm 0.3	18.6 \pm 0.5	31.8
Half life (h)	0.6	0.6	1.1

Neonate parameters were provided as means only because different animals were used for each time point- the same animals could not be studied sequentially due to their small blood volumes. In the adults, pharmacokinetic parameters were similar between the two dose groups with the obvious exception of AUC. The neonates did not clear drug as rapidly, leading to longer half life and greater AUC for the same 20 mg/kg dose that the adults received. The investigators attributed this to the lower capacity of neonates to metabolize trovafloxacin via phase II conjugation enzymes not yet fully developed in these young rats.

Pharmacokinetics of Trovafloxacin in Beagle Dogs Following Single Intravenous Administration of Alatrofloxacin or Trovafloxacin and Single Oral Administration of Trovafloxacin 23-099219-1)

Alatrofloxacin mesylate was administered intravenously to 3 male beagle dogs via the cephalic vein as a bolus dose of 5 mg/kg. Blood samples were collected 3 minutes to 2 hours after dosing and were immediately acidified with KH_2PO_4 to prevent hydrolysis of alatrofloxacin to trovafloxacin. Alatrofloxacin was detected in plasma only at the 3 minute time point (mean concentration $2.80 \pm 1.27 \mu\text{g/ml}$). It was not detected in subsequent samples, the next of which was drawn 10 minutes after dosing. Alatrofloxacin appears to be rapidly hydrolyzed (loss of 2 L-alanine groups) to trovafloxacin in the dog. A third compound, a mono-L-alanine analog of trovafloxacin, was also detected in plasma 3 minutes after the IV bolus dose of alatrofloxacin and demonstrates a sort of midway point between the conversion of alatrofloxacin (which has 2 L-alanine groups) to trovafloxacin.

A 8-9 minute IV infusion of 10 mg/kg alatrofloxacin mesylate was given to 1 male and 3 female beagles and blood samples were drawn 0 - 8 hours after the beginning of dosing. For the 10 mg/kg IV alatrofloxacin dose, the mean clearance of trovafloxacin was $7.7 \pm 1.1 \text{ ml/min/kg}$, the volume of distribution was $1.5 \pm 0.6 \text{ l/kg}$, the AUC was $22.0 \pm 3.3 \mu\text{g}\cdot\text{hr/ml}$ and the half life was calculated to be 2.0 h.

A 5 minute IV infusion of 5 mg/kg trovafloxacin mesylate was given to 3 male and 3 female beagle dogs. Blood samples were drawn 0 - 8 hours after the end of infusion. One week later, the same dogs were given an oral dose of 20 mg/kg trovafloxacin mesylate in 0.5% methylcellulose and blood was obtained 0 - 12 hours after dosing.

	IV 5 mg/kg	Oral 20 mg/kg
Clearance (ml/min/kg)	11.1 ± 3.0	---
Volume of Distribution (l/kg)	1.7 ± 0.5	---
AUC ($\mu\text{g}\cdot\text{hr}/\text{ml}$)	8.0 ± 1.8	17.7 ± 9.0
Half life (h)	1.7	2.4

The mean absolute bioavailability of the oral 20 mg/kg dose of trovafloxacin was $58 \pm 41\%$. The variability among the dogs (range of 17-115%) may have been due to emesis. The C_{max} of trovafloxacin after the oral 20 mg/kg dose was $3.5 \pm 1.5 \mu\text{g}/\text{ml}$ and the T_{max} was 2.3 ± 1.2 hours. The half lives of 10 mg/kg IV alatrofloxacin, 5 mg/kg IV trovafloxacin, and 20 mg/kg oral trovafloxacin were similar (1.7 - 2.4 hours) and the volume of distribution ($1.5 - 1.7 \text{ l/kg}$) for trovafloxacin was similar whether it was given IV as alatrofloxacin or trovafloxacin.

Pharmacokinetics of Trovafloxacin in Cynomolgus Monkeys Following Single Intravenous Administration of Alatrofloxacin or Trovafloxacin and Single Oral Administration of Trovafloxacin (93-099219-2)

Alatrofloxacin hydrochloride was administered as an IV bolus at a dose of 20 mg/kg to 2 male cynomolgus monkeys. Blood samples were collected 0 - 24 hours after dosing and immediately acidified with KH_2PO_4 to prevent hydrolysis of alatrofloxacin to trovafloxacin. Although alatrofloxacin could not be detected at any time point, the mono L-alanine form of trovafloxacin was detected at the 0.17 and 0.33 hour time points. The mono L-alanine form of trovafloxacin appears to be an intermediate between alatrofloxacin (which contains 2 L-alanine groups) and trovafloxacin. The investigators believe that the L-alanine groups are sequentially hydrolyzed in the plasma; this seems to be a reasonable assumption. For the 20 mg/kg IV alatrofloxacin hydrochloride dose, the mean clearance of trovafloxacin was $7.6 \text{ ml}/\text{min}/\text{kg}$, the volume of distribution was 4.0 l/kg , the AUC was $43.8 \mu\text{g}\cdot\text{hr}/\text{ml}$ and the half life was calculated to be 5.9 h.

A 5 minute IV infusion of 20 mg/kg trovafloxacin mesylate was given to 3 male cynomolgus monkeys. Blood samples were drawn 0 - 24 hours after the end of infusion. Pharmacokinetic parameters were similar to those observed following the alatrofloxacin hydrochloride infusion. For the 20 mg/kg IV trovafloxacin mesylate dose, the mean clearance of trovafloxacin was $7.2 \pm 1.1 \text{ ml}/\text{min}/\text{kg}$, the volume of distribution was $4.3 \pm 2.3 \text{ l/kg}$, the AUC was $47.1 \pm 7.2 \mu\text{g}\cdot\text{hr}/\text{ml}$ and the half life was calculated to be 5.8 h.

Another group of 3 male cynomolgus monkeys was given an oral dose of 20 mg/kg trovafloxacin mesylate in deionized water and blood was drawn 0 - 48 hours after dosing. For the 20 mg/kg oral dose, C_{max} was $4.1 \pm 1.9 \mu\text{g}/\text{ml}$, T_{max} was $4.1 \pm 2.3 \text{ h}$, AUC was $54.3 \pm 23.1 \mu\text{g}\cdot\text{hr}/\text{ml}$, and the calculated half life was 9.1 hours. Absolute bioavailability (comparison made to the previous set of monkeys) was 115%, indicating complete absorption of 20 mg/kg oral dose by this species.

Pharmacokinetics of Trovafloxacin in New Zealand White Rabbits Following Single Intravenous or Oral Administration of Trovafloxacin 96-099219-4)

A 3-5 minute infusion of 40 mg/kg trovafloxacin mesylate was administered to 6 male New Zealand White rabbits and blood samples were collected 0.25 - 8 hours after dosing. For this dose of trovafloxacin, the mean clearance was 29.5 ± 11.6 ml/min/kg, the volume of distribution was 2.8 ± 0.8 l/kg, the AUC was 29.5 ± 15.4 $\mu\text{g}\cdot\text{hr}/\text{ml}$ and the half life was calculated to be 1.7 h.

An oral dose of 200 mg/kg trovafloxacin mesylate in 0.5% methylcellulose was given to 3 female New Zealand White rabbits and blood samples were collected 0.5 - 24 hours after dosing. For this dose of trovafloxacin, C_{max} was 4.6 ± 0.3 $\mu\text{g}/\text{ml}$, T_{max} was 1.2 ± 0.8 h, AUC was 24.1 ± 8.4 $\mu\text{g}\cdot\text{hr}/\text{ml}$, and the calculated half life was 2.6 hours. Absolute bioavailability (comparison made to the previous set of rabbits) was 16% (range of 13 -23%). The longer half-life of the drug after oral administration, compared to IV, was believed to be due to prolonged absorption of trovafloxacin because of its low aqueous solubility.

Protein Binding of Trovafloxacin:**Serum Protein Binding of Trovafloxacin in Rats, Rabbits, Dogs, Monkeys and Humans 92-099219-5)**

Fresh serum obtained from rats, rabbits, dogs, monkeys, and humans was used to determine the protein binding capacity of radiolabeled trovafloxacin via 30 minute equilibrium dialysis across Spectra/Por membranes into 0.067 M sodium phosphate buffer (pH 7.44). The mean percentages (duplicates) of unbound trovafloxacin were:

Species	1 $\mu\text{g}/\text{ml}$ Trova	5 $\mu\text{g}/\text{ml}$ Trova
Rat	< 11 %	3 %
Rabbit	27 %	27 %
Dog	25 %	24 %
Monkey	35 %	33 %
Human	30 %	28 %

Protein binding was similar within species no matter which concentration of trovafloxacin was used. The percentage of unbound trovafloxacin was similar in serum from rabbit, dog, monkey, and human. The percentage of unbound trovafloxacin in rat serum was lower than for the other species.

Tissue Distribution:**Blood/Plasma Ratios of Trovafloxacin in Rats, Dogs, Monkeys and Humans 93-099219-3)**

Fresh blood from Sprague-Dawley rats, beagle dogs, cynomolgus monkeys, and humans was incubated for 20 minutes at 37°C with 0.01 μCi of ^{14}C -trovafloxacin mesylate

(99% radiochemical purity, 47.7 $\mu\text{Ci}/\text{mg}$) plus enough "cold" drug to give final trovafloxacin concentrations of 0.5, 5.0 and 20.0 $\mu\text{g}/\text{ml}$. After incubation, blood was centrifuged to separate plasma and erythrocytes and the amounts of radiolabel determined in each. Trovafloxacin did not appear to be preferentially distributed into the red blood cells of any of the species tested (the fraction distributed to rat and human red blood cells was even lower than that distributed to red cells of the monkey and dog). Results of the test did not differ when trovafloxacin concentrations in the blood were varied, so results of the experiments were averaged. The blood/plasma ratios of trovafloxacin in the rat, dog, monkey, and human were 0.84, 0.94, 1.04, and 0.74, respectively. The fractions of trovafloxacin distributed to the red blood cells of these species were 0.29, 0.45, 0.41, and 0.19, respectively.

Distribution of [^{14}C] Trovafloxacin into Gastric Tissue of Swiss-Webster Mice Following Intravenous Administration (94-099219-3)

Swiss-Webster mice (5 per time point) were given an intravenous bolus dose of 10 mg/kg trovafloxacin (mix of $^{14}\text{-C}$ and "cold" drug) through the tail vein. Animals were sacrificed 1, 2, 4, 6, and 24 hours after dosing and the concentrations of trovafloxacin in the serum and gastric tissues were measured. did not detect metabolites of trovafloxacin in the samples, so liquid scintillation counting was predominantly relied upon. Trovafloxacin distributed to gastric tissues in the mice, with a higher level in the gastric mucosa than the gastric submucosa. At the time points used for this study, the concentrations of trovafloxacin in the gastric tissues tended to be higher than the serum concentration.

Tissue Distribution of Trovafloxacin in Beagle Dogs Following Oral Administration of Multiple Doses (93-099219-5)

Two male beagle dogs received 10 mg/kg daily oral doses of trovafloxacin for 5 days. Two hours after the final dose of drug, animals were sacrificed and tissues harvested. with UV detection was used to determine mean trovafloxacin concentrations in the following:

	$\mu\text{g}/\text{g}$ or $\mu\text{g}/\text{ml}$	Ratio with Serum
Serum	3.9	---
Urine	25	6.4
Cerebrospinal Fluid	0.7	0.2
Bile	254	65
Lung	3.5	0.9
Liver	18.4	4.7
Heart	7.5	1.9
Kidney	7.8	2.0
Brain	1.7	0.4
Skeletal Muscle	6.2	1.6
Fat (White)	0.6	0.1

Results are consistent with biliary and urinary excretion of trovafloxacin and good

tissue penetration of the drug. Distribution of trovafloxacin to the brain and CSF was less than that for most of the other tissues measured except for fat.

Whole-Body Autoradioluminography of Female and Male Long-Evans Rats After Single Intravenous Administration of [^{14}C] Trovafloxacin 94-099219-1)

Fasted male and female Long Evans rats received intravenous infusions of 10 mg/kg unradiolabeled and ^{14}C -trovafloxacin. Rats were sacrificed 1, 4, 8, and 168 hours after dosing and frozen immediately in a hexane-dry ice bath. Carcasses were embedded in 3% CMC and 25 mm sections were cut from various levels. Sections were placed in phosphor cassettes for 8 days before the imaging screens in the cassettes were scanned using

One hour after infusion, radioactivity could be observed in most tissues with the highest levels in the GI tract. In the eye, the uvea contained about 30 times more radioactivity than the vitreous humor. The brain and spinal cord contained less radioactivity than the blood. The amount of radioactivity in all tissues except for the cecum and uvea were lower at the 4 hour time point than they had been 1 hour after infusion. Levels of radioactivity in all tissues fell further 8 and 168 hours after infusion. At the 8 hour time point, the GI tract, uvea, and skin contained the greatest amounts of radioactivity. At the 168 hour time point, radioactivity was still detectable in these tissues and also in the arterial wall, pineal gland, and liver. As has been demonstrated with other quinolones, trovafloxacin appears to be associated with melanin.

Whole-Body Autoradioluminography of Female and Male Long-Evans Rats After Single Oral Administration of [^{14}C] Trovafloxacin 95-099219-1)

The study immediately above was repeated using an oral dose of 10 mg/kg trovafloxacin dissolved in 0.5% methylcellulose. Rats were sacrificed 1, 4, 8, 100, 300, and 600 hours after dosing. The investigators were particularly interested in estimating the half life of trovafloxacin-associated radioactivity in melanin-rich tissues. The GI tract had the highest levels of radioactivity 1 hour after dosing, but levels in the stomach and small intestine fell to undetectable levels 8 hours after dosing. In the cecum, peak radioactivity occurred 4 hours after dosing, had declined by 8 hours after dosing, and was not detected beyond the 100 hour time point. Radioactivity was detected in the pineal gland, but not other cerebral tissue, 1, 4, and 8 (males only) hours after dosing, but not later. Most tissues in the body (kidney cortex, kidney medulla, heart, liver, lung, muscle, spleen) peaked 1 hour after dosing and declined over the next 7 hours. Radioactivity was detected in the uvea (highest in the ciliary body, then choroid, then iris) 1 hour after dosing with a peak observed at the 8 hour time point. Levels of radioactivity in the components of the uvea slowly declined throughout the rest of the study, but could still be detected 600 hours after dosing. The half life of trovafloxacin-associated radioactivity in the uvea was estimated to be 23 days in the female pigmented rat and 15 days in the males. The levels of radioactivity in pigmented skin were greater than those in non-pigmented skin. Radioactivity could be detected in pigmented skin up to 100 hours after dosing, but was not detected in nonpigmented skin after the 1 hour time point in females or the 4 hour time point in males. The presence of radioactivity in blood vessel walls and in intravertebral discs may indicate an affinity for elastin as well as melanin.

Whole-Body Autoradioluminography of Female and Male Long-Evans Rats After Single Intravenous Administration of [¹⁴C] Alatrofloxacin 96-116517-2)

The studies immediately above were repeated with ¹⁴C-Alatrofloxacin mesylate. Alatrofloxacin was infused into the jugular veins of Long Evans rats at a dose of 15.7 mg/kg and the animals sacrificed 0.25, 1, 4, 8, 100, 300, and 600 hours after dosing. Amounts of radioactivity in frozen sections of the rats were consistent with those observed in the studies with intravenously and orally administered radiolabelled trovafloxacin.

Metabolism and Excretion:**Identification of Trovafloxacin Metabolites in Human Bile Following Oral Administration of Trovafloxacin 196-099219-2)**

Bile samples were collected from a human subject 12-16 hours after a single oral trovafloxacin dose of 200 mg. was used to identify free trovafloxacin, trovafloxacin glucuronide (the primary metabolite found in bile), and trovafloxacin sulfate. A peak corresponding with N-acetyltrovafloxacin was barely detectable.

Excretion and Metabolism of Trovafloxacin in Man 96-099219-1)

A single oral dose of ¹⁴C-trovafloxacin (200 mg) was given to healthy human male volunteers. Urine and feces were collected for the next 240 hours (24 hour sampling intervals) and blood was drawn 0.5, 1, 2, 4, 8, 12, 24, 36, 48, 72, 96, 120, 144, 168, 192, 216, and 240 hours after dosing. Total radioactivity was measured in blood, urine, and homogenized feces (b)(4) was used to separate trovafloxacin and its metabolites in these biological samples and was used for identification. The major route of excretion of trovafloxacin-associated radioactivity over 240 hours was the feces ($63.27 \pm 2.21\%$ of the dose) and most of it was parent drug (43.25% of the dose). Approximately $23.05 \pm 3.69\%$ of the radioactive dose was excreted in urine, with trovafloxacin glucuronide as the major portion (12.75% of the dose). Other metabolites detected in urine were N-acetyl trovafloxacin (0.42% of dose), unchanged trovafloxacin (5.9% of dose), trovafloxacin N-sulfate conjugate (0.21% of dose), M 12 (0.16% of dose), M8 (0.16% of dose), and M6 (an acetylated trovafloxacin, 1.18% of dose). Other metabolites detected in feces were trovafloxacin N-sulfate conjugate (3.89% of dose), M12 (0.88% of dose), and M6 (9.19% of dose). The mean C_{max} of trovafloxacin was 2.85 µg/ml and it occurred about 1.25 ± 0.5 hours after dosing. The terminal half life of the drug was 13.59 hours and the mean AUC was 32.18 ± 8.63 µg·hr/ml. In the first 4 hours of sampling, the parent drug accounted for 90% of the drug in serum and from over the next 20 hours. Metabolites detected in serum included 2 ester glucuronides and acetylated trovafloxacin.

In Vitro Hydrolysis of CP-116,517 to CP-99-219 in Neonatal and Adult Rat, Guinea Pig, Dog, Monkey and Human Serum 193-116517-1)

Fresh serum obtained from neonatal rats, adult rats, guinea pigs, dogs, monkeys, and

humans was used to determine the rate of alatrofloxacin hydrolysis to trovafloxacin *in vitro*. Alatrofloxacin was added to give final concentrations of 75-100 $\mu\text{g/ml}$ (based on free trovafloxacin equivalents) and serum was incubated for 1-5 hours at 37°C. Aliquots of serum were taken at various times after the start of incubation and acidified with KH_2PO_4 to prevent further hydrolysis of alatrofloxacin. detection was used to measure the concentrations of alatrofloxacin, trovafloxacin, and a mono L-alanine intermediate. Hydrolysis of alatrofloxacin occurred rapidly in all species with half lives less than 3 minutes in neonatal and adult rats, guinea pigs, and humans. Alatrofloxacin half lives in dog and monkey were still relatively short, at about 21 and 9 minutes, respectively. It should be noted that dog data generated with alatrofloxacin *in vivo* (above) suggested an even shorter half life for alatrofloxacin hydrolysis. Rapid formation and disappearance of the mono-L-alanyl intermediate suggested sequential hydrolysis of the L-alanyl groups.

In Vitro Metabolism of [^{14}C] Trovafloxacin by Rat and Human Liver Slices 94-099219-4)

Rat or human liver slices were incubated at 37°C in Henseliet buffer (pH 7.5) with ^{14}C -trovafloxacin at a concentration of 15 $\mu\text{g/ml}$ for 15-240 minutes (rat) or 60-240 minutes (human). Over time, the amount of radioactivity in the buffer decreased while the amount in the liver slices increased. HPLC analysis of the buffer and slice homogenates showed that the majority of the radioactivity in both rat and human preparations was unchanged trovafloxacin (95%). Measurable amounts of N-acetyl-trovafloxacin were detected in both preparations after 120 minutes. A smaller amount of N-acetyl, acyl glucuronide conjugate of trovafloxacin was detected in the rat preparations after 60 minutes, but not in the human slices or incubation buffer.

Excretion and Metabolism of Trovafloxacin in Sprague-Dawley Rats 94-099219-2)

^{14}C -trovafloxacin mesylate was given to 6 male and 6 female fasted Sprague-Dawley rats as a single oral dose of 10 mg/kg as a suspension in 0.1% methylcellulose. Half of the rats underwent bile duct cannulation; all were housed in metabolism cages for collection of urine and feces. Animals were fed 4 hours after dosing and given access to 5% sucrose solution during the whole study.

Another 12 fasted male rats were given an oral dose of 50 mg/kg ^{14}C -trovafloxacin mesylate. These rats were fed 6 hours after dosing and had free access to water. Blood was collected from 2 rats per time point 0, 1, 2, 4, 6, 8, and 24 hours after dosing. These samples were used to quantify the amounts of trovafloxacin and its metabolites in serum.

trovafloxacin from its metabolites in the biological samples collected above.

The major route of excretion of trovafloxacin in the noncannulated rats after a 10 mg/kg oral dose was fecal, with a mean of $97.9 \pm 5.9\%$ of the dose recovered in males and $96.3 \pm 1.0\%$ of the dose recovered in females in 96 hours (with the majority recovered within the first 24 hours). The mean percentages of the dose excreted in urine were $4.4 \pm 2.6\%$ and $4.1 \pm 0.6\%$, respectively. In the bile duct cannulated rats, about 60% of the dose was

recovered in the bile of male and female over 96 hours. In the males, $9.3 \pm 9.4\%$ was recovered in urine and $28.2 \pm 15.2\%$ was recovered in feces. In the females, recovery was not as complete with $7.0 \pm 4.7\%$ of the dose in urine and $5.4 \pm 3.9\%$ in feces.

Several glucuronide conjugates were detected in bile from the male and female rats. They were believed to be ester glucuronides as they were resistant to the action of β -glucuronidase. Together, they accounted for the majority of the bile radioactivity.

Another major peak in the bile was identified as a N-acetyl derivative of trovafloxacin. Unchanged trovafloxacin was also found in bile as was a sulfamate derivative of trovafloxacin, and a glycine conjugate of N-acetyl trovafloxacin. Several of these metabolites were also found in urine of cannulated and noncannulated rats: glucuronides- associated with 90% of radioactivity in the urine, unchanged trovafloxacin, N-acetyl trovafloxacin, and a trace of trovafloxacin sulfate conjugate. The feces of noncannulated rats contained predominantly N-acetyl trovafloxacin (about 62%) and unchanged trovafloxacin (about 15%), with a trace of the sulfate conjugate. N-acetyl trovafloxacin was not detected in feces from bile-duct cannulated rats.

The metabolites of trovafloxacin found in serum pooled from samples drawn 1-24 hours after dosing were an ester glucuronide of N-acetyl trovafloxacin (16% of radioactivity), a trace of ester glucuronide of trovafloxacin, N-acetyl trovafloxacin (18% of radioactivity). About 55% of the radioactivity of the pooled serum was associated with unchanged trovafloxacin.

The data indicate that the major route of elimination of trovafloxacin in Sprague-Dawley rats is fecal, with major biliary excretion.

Excretion and Metabolism of Trovafloxacin in Beagle Dogs 94-099219-2)

^{14}C -trovafloxacin mesylate was given to 4 male and 4 female fasted beagle dogs as a single oral dose of 10 mg/kg as a suspension in 0.1% methylcellulose. Half of the dogs underwent bile duct cannulation; all were housed in metabolism cages for collection of urine and feces. The dogs were fed 4 hours after dosing and had free access to water. Blood samples were collected from cannulated dogs 0, 1, 2, 4, 6, 24, 48, 72, and 96 hours after dosing and from noncannulated dogs 1 and 4 hours after dosing.

trovafloxacin from its metabolites in the biological samples collected above.

In noncannulated dogs, the main route of trovafloxacin excretion after a 10 mg/kg oral dose was fecal, with a mean of 97.0% of the dose recovered in males and 98.5% of the dose recovered in females in 96 hours. Mean urinary excretion in these dogs was 4.3% for males and 1.1% for females over 96 hours. In the cannulated dogs, 40.0% and 51.8% of the dose was excreted in the urine of males and females, respectively, over the same time period. The bile contained 41.0% (males) and 15.7% (females) of the dose and the feces contained 15.9% (males) and 30.3% (females).

Metabolites were recovered in bile, urine, feces, and blood as follows:

**Trovafloxacin Metabolites in Bile Duct Cannulated and Non-Cannulated Beagles
After a Single Oral Dose of 10 mg/kg (Mean % Total Dose for Males and Females)**

Metabolite	Bile	Urine		Feces	
		Can	Non	Can	Non
Ester Glucuronide	11.5	10.6	ND*	ND	ND
Unchanged Trovafloxacin	6.4	19.8	1.31	13.6	59.0
Pyrroline Ester Glucuronide + Diacid + Hydroxy Carboxylic Acid (Co-Eluted)	1.9	1.4	0.5	ND	Trace
N-Acetyl Trova + Amide (Co-Eluted)	0.15	1.1	Trace	ND	23.7 [#]
Cyclopropanone	0.4	ND	ND	ND	ND
Pyrroline	1.2	Trace	ND	ND	Trace

*Not Detected

[#]All radioactivity accounted for by N-Acetyl-trovafloxacin in feces of noncannulated dogs

The metabolites listed in the table above (except for the cyclopropanone which was not detected in the serum of noncannulated dogs) were also detected in serum of cannulated and noncannulated dogs. The majority of radioactivity circulating in the serum was associated with unchanged trovafloxacin (55% of serum radioactivity). The metabolites each accounted for of serum radioactivity; the trovafloxacin ester glucuronide was highest at about 10.5%.

The data indicate that in noncannulated dogs, most of a 10 mg/kg trovafloxacin dose was eliminated in the feces. Biliary excretion of trovafloxacin occurred in the dog, particularly of the unchanged drug and its ester glucuronide. *In vitro* studies using freshly harvested dog cecae suggested that the intestinal microflora in these animals were primarily responsible for the production of N-acetyl-trovafloxacin. A significant amount of this metabolite was found in the feces of noncannulated dogs. Oxidative metabolic pathways did not appear to play a major role in trovafloxacin metabolism in the dog as relatively small amounts of oxidized trovafloxacin metabolites were found in the blood and excreta.

Metabolism and Excretion of Alatrofloxacin in Sprague-Dawley Rats (96-116517-1)

¹⁴C-alatrofloxacin mesylate was infused into the jugular vein of 6 male and 6 female Sprague-Dawley rats at a dose level of 10 mg/kg. Two rats per gender underwent bile duct cannulation; all were housed in metabolism cages for collection of urine and feces. Bile was

collected in an ammonium formate buffer (pH 3) to prevent alatrofloxacin hydrolysis. The rats were fed 4 hours after dosing and had free access to water. Blood samples were collected from noncannulated rats 0, 0.03, 0.08, 0.17, 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, and 24 hours after dosing.

trovafloxacin from its metabolites in the biological samples collected above.

Over 96 hours, 96.1% of the radioactivity was excreted in the feces and 4.9% in the urine of noncannulated rats. In the bile duct cannulated rats, of the radioactive dose was recovered in the bile during the first 8 hours of collection. The bile contained 2 metabolites that were not detected in blood, feces, or urine: a glucuronide conjugate of trovafloxacin (16% of bile radioactivity) and a glucuronide conjugate of N-acetyl-trovafloxacin (51% of bile radioactivity). N-acetyl-trovafloxacin was identified in bile (12.6% of bile radioactivity) of cannulated rats, and in the serum (8% of serum radioactivity), urine (3.1% of dose) and feces (60% of dose) in noncannulated rats. Trovafloxacin was found in bile (1% of bile radioactivity) of cannulated rats, and in the serum (81% of serum radioactivity), urine (0.9% of dose), and feces (21.5% of dose) of noncannulated rats. The mono L-alanine trovafloxacin (an intermediate of alatrofloxacin conversion to trovafloxacin) was identified in bile (12.5% of bile radioactivity) and serum (5.7% of serum radioactivity). A trace of unchanged alatrofloxacin was also detected in bile and serum. Bile contained two other radioactive peaks that could not be elucidated, but they accounted for a relatively small portion of bile radioactivity.

Alatrofloxacin is rapidly converted to trovafloxacin in the blood of Sprague-Dawley rats. Its metabolic and excretion profile are consequently similar to that of trovafloxacin. The primary route of elimination was in the feces and extensive biliary excretion occurred. Glucuronidation was a major metabolic pathway in the rats.

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ON 10/10/01

SUMMARY AND EVALUATION:

Trovafloxacin is well absorbed from the GI tract in animals and man. Absolute bioavailability in humans, cynomolgus monkeys, dogs, and rats is about 88%, 115%, 58% (estimate may be low due to vomiting in some dogs), and 67%, respectively. Bioavailability in the rabbit is much lower, about 16%. Protein binding is moderate (about 70%) in most species, including humans, but is greater in the rat ($\geq 90\%$). Alatrofloxacin is rapidly converted to trovafloxacin in the blood upon intravenous infusion. Metabolism and elimination of both trovafloxacin and alatrofloxacin are similar, with the feces as the main route of elimination in humans, rats, and dogs. Biliary excretion of trovafloxacin is extensive in rats and is also significant in dogs and humans. Glucuronidation appears to be a major metabolic pathway for trovafloxacin in rats, dogs, and humans, with much less oxidative metabolism in these species. Unchanged trovafloxacin was the most abundant form of the drug found in the serum of rats, dogs, and humans. The plasma half life of the drug in most animal species studied was much shorter than for humans. Trovafloxacin half life in dogs, rats, and rabbits was 2-2.5 hours after a 5-40 mg/kg oral dose, 9.1 hours in monkeys after a 20 mg/kg oral dose and 13.6 hours in humans after a 200 mg total oral dose. Like most fluoroquinolones, trovafloxacin is widely distributed to vascular tissues after oral or IV administration (although distribution into the brain and spinal cord was less than most other tissues) and appears to have an affinity for melanin.

Single oral doses of trovafloxacin were relatively nontoxic. The minimum lethal oral dose was between 1000-2000 mg/kg in both mice and rats and the clinical signs observed included decreased activity and respiration, ptosis, ataxia and piloerection. The rat that died at

this dose level experienced clonic convulsions with death occurring 4 hours after administration of trovafloxacin. Mortality was delayed in the mice, occurring 7-8 days after dosing. The minimum lethal intraperitoneal dose of trovafloxacin was 100-250 mg/kg for mice and 250-500 mg/kg for rats. Clinical signs similar to those observed following oral dosing were reported with the additional observation of writhing, perhaps indicative of discomfort at the site of injection.

The minimum lethal single intravenous dose of alatrofloxacin was between 50-125 mg/kg for mice and greater than 75 mg/kg (the highest dose tested) for rats. Clinical signs observed in the mice included exophthalmia, "jittery appearance", ataxia, vocalization, decreased activity and respiration, darkening eyeballs, flushed feet, ptosis, tremors, straub tail, and clonic convulsions. Mortality occurred within 10 minutes after dosing. Clinical signs in the rats included "jittery appearance", ataxia, decreased activity and respiration, flushing of ears and feet, exophthalmia, and ptosis.

No mortality was observed in rats given 50-200 mg/kg of trovafloxacin via oral gavage for 2 weeks. No treatment-related gross or histopathologic changes were seen at necropsy, but there were slight reductions in hemoglobin in high dose females and in total serum protein in mid (100 mg/kg) and high dose males. There was also a slight suppression of body weight gain in the high dose males; the mean body weight of these rats was about 7% less than control at the end of the study. Slight reductions in serum magnesium observed in most of the animals were believed to have been due to chelation of Mg^{++} ion by trovafloxacin. Rats given trovafloxacin via oral gavage for one month at doses of 25-200 mg/kg experienced no mortality. Again, no treatment-related gross or histopathologic changes were seen at necropsy. The only clinical sign noted was salivation in most high dose rats. Reduction in body weight gain was observed in the high dose males, but no reduction in food consumption was seen. Small reductions in serum albumin and globulin were seen in high dose males and mid (75 mg/kg) and high dose females, but the values remained in the normal range for rats. Slight reductions in hematocrit and hemoglobin were observed in rats from the high dose group, but also remained in the normal range. The NOAEL in this 4 week study was 75 mg/kg for males (based upon the reduction in body weight gain at the highest dose) and 200 mg/kg for females. No drug-related mortality was observed in rats given trovafloxacin via oral gavage daily for 6 months at 25-200 mg/kg. Salivation was observed in rats from each dose group; the incidence increased with dose. Inhibition of body weight gain was observed in the high dose males. Hematocrit and hemoglobin were slightly lower in trovafloxacin-treated male rats compared to controls. Mean serum protein and globulin concentrations were less in high dose rats than controls. A dose-related increase in the incidence of minimal to mild "fatty change" was seen in the livers of male rats from all trovafloxacin groups. Tubular degeneration of the testes was observed in 1-2 rats from each trovafloxacin group and is considered drug-related despite the relatively low incidence. The NOAEL in the 6 month study was 200 mg/kg for the female rats, but was not determined in males due to the histopathologic observations in the liver and testes.

Two (one male, one female) out of 4 rats receiving intravenous alatrofloxacin at 100 mg/kg during a 2 week study died within 5 minutes after the first dose. All 4 rats experienced tonic/clonic convulsions. The survivors, and the remaining 4 mice in this dose group received 75 mg/kg of alatrofloxacin for the remainder of the study. Rats that received 50 or 75 mg/kg of alatrofloxacin demonstrated clinical signs such as salivation, hypoactivity, prostration. Rats

in the 75 mg/kg group also experienced dyspnea and generalized erythema. No clinical signs were observed at 20 mg/kg, the NOAEL for this study. Drug-related gross or histopathologic changes were not observed upon necropsy. In a one month intravenous alatrofloxacin study, no trovafloxacin-related mortality was observed at 6.5, 20, or 50 mg/kg. Hypoactivity, labored breathing, sternal recumbency, erythema and salivation were observed in the high dose group. Slight reductions in hematocrit and hemoglobin were seen in the high dose rats and a slight reduction in serum magnesium was detected in the high dose females. Drug-related gross or histopathologic changes were not observed in the high dose rats; tissues from the 2 lower dose groups were not examined microscopically.

Neonatal rats were more sensitive to trovafloxacin-induced tremors than adults or older pups. This may be due to the younger animals having less developed blood brain barriers; therefore, higher trovafloxacin concentrations are achieved in the brain.

Studies in mice indicated that biphenyl acetic acid, an active metabolite of the nonsteroidal anti-inflammatory drug fenbufen, did not enhance the capacity of either oral trovafloxacin or intravenous alatrofloxacin to cause convulsions in these animals. The pro-convulsant properties of several other quinolones, particularly enoxacin and norfloxacin, are enhanced by fenbufen or its metabolite.

No mortality was observed in beagles dosed orally with 12.5-50 mg/kg of trovafloxacin for 14 days. Due to emesis, the high dose was administered as 25 mg/kg BID. This lowered the frequency of emesis. Erythema, salivation, and hypoactivity were also observed in the high dose group. Glomerular hypoplasia was observed in the kidneys of both high dose dogs; however, the effect was not observed at this dose in a subsequent study with a longer period of administration. When trovafloxacin was given orally to beagles for one month at 5, 15 (7.5 BID), or 50 (25 BID) mg/kg, no dogs died. Emesis occurred occasionally in the mid and high dose groups, erythema of the ears and salivation were seen in the high dose dogs, and decreased activity was seen in all dose groups. Slight reductions in serum globulins were observed in high dose males and all females. Erosion of articular cartilage at the knee joint was observed in one high dose male. The NOAEL in this study was 5 mg/kg based upon the observation of emesis, but was 15 mg/kg if emesis is not considered. In a 6-month study, mortality was not observed at doses of 7.5, 15 (7.5 BID), or 50 (25 BID) mg/kg. General or localized erythema was observed in all dose groups and salivation and emesis were observed in dogs from the highest dose group. Microscopic examination of the liver revealed hepatocellular vacuolar degeneration and necrosis in 2/8 (1 male and 1 female) dogs from the high dose group and both animals had elevated serum liver enzymes. Testicular degeneration (slight or mild vacuolation of germinal epithelium, multinucleated giant cells in seminiferous tubules) was seen in 4/4 high dose male dogs and has been observed in animals treated with other quinolones. The NOAEL in this 6-month beagle study was 15 mg/kg. In a subsequent study, beagles were given 50 mg/kg/day of trovafloxacin via the oral route until their serum liver enzymes (measured every 2 weeks) were 3X greater than baseline or for up to 6 months if no liver enzyme elevations were detected. Liver biopsies were taken prior to the study, when 3-fold increases in serum liver enzymes were observed, and randomly during the study in dogs showing no increase. Dosing with trovafloxacin was discontinued in 3/16 dogs demonstrating 3-fold increased in liver enzymes whose biopsies revealed hepatocellular changes. An additional dog demonstrated elevated liver enzymes with no evidence of necrotizing hepatocellular inflammation. This animal continued trovafloxacin treatment and

the serum liver enzyme levels fell over time. All animals were biopsied again at the end of 6 months and liver tissue was collected at necropsy from some controls and all drug-treated dogs for microscopic evaluation. No dogs without elevated hepatic enzyme elevations demonstrated necrotizing hepatocellular inflammation. Data from this study indicated that elevation in liver enzymes, especially ALT, accurately predicted the presence of necrotizing inflammation of periportal hepatocytes. The necrotic changes were no longer evident approximately 2 months after discontinuation of the drug.

Intravenous doses of alatrofloxacin at 2, 10, or 20 mg/kg administered daily for 10 days did not cause mortality in beagle dogs. Animals receiving the mid and high doses demonstrated dose-related salivation and emesis. Erythema was observed in animals from all dose groups. Only sporadic salivation and erythema were observed in the 2 mg/kg group. In a one month study of intravenous alatrofloxacin at the same dose levels, convulsions were observed in the 20 mg/kg group and the dose level was dropped in steps to 15, then 12 mg/kg. One female in the high dose group received 10 mg/kg for most of the study due to convulsions at 12 mg/kg. The incidence of convulsions fell as the high dose was lowered, but tremors were still observed in this group. No mortality occurred during the study. Dose-related clinical signs observed in the high and mid dose dogs included small muscle contractions, salivation, emesis (incidence decreased after first week of dosing), cutaneous erythema, and hypoactivity. Erythema, salivation, and emesis were sporadically observed in the low dose group as well. Microscopic examination of the tissues revealed no apparent alatrofloxacin-related lesions. On the basis of the CNS effects observed in the 2 higher dose groups, the NOAEL for alatrofloxacin in this one month study was 2 mg/kg/day.

Orally or subcutaneously administered trovafloxacin was not antigenic in guinea pigs; however, subcutaneous alatrofloxacin was antigenic in this animal model.

The phototoxic potential of single oral doses of trovafloxacin was less than that of lomefloxacin in female BALB/c mice exposed to ultraviolet A radiation, even at trovafloxacin doses as high as 250 mg/kg where signs of systemic toxicity were observed (reduced activity, tremors). Phototoxic reactions after 250 mg/kg of trovafloxacin were described as well-defined erythema of the ears. At 90 mg/kg, phototoxicity was still evident, but less than that observed at 250 mg/kg. The reviewer's NOEL for trovafloxacin-induced phototoxicity in this study was 10 mg/kg as slight evidence of phototoxicity was observed at 30 mg/kg. Oral doses of trovafloxacin up to 300 mg/kg administered for approximately 8 weeks in conjunction with exposure to simulated sunlight were not phototoxic to hairless mice. Trovafloxacin-related mortality was observed at doses ≥ 100 mg/kg.

Like other members of the quinolone class of antimicrobials, trovafloxacin will induce cartilage lesions in the joints of juvenile animals. Erosion of articular cartilage was observed in the knee joint of a dog (4-6 months old) that received 50 mg/kg (25 mg BID) for 4 weeks.

Trovafloxacin did not induce reverse mutation at the *his* locus of *S. typhimurium* strains TA 98, TA 100, TA 1535, and TA 1537 in the presence or absence of metabolic activation. A similar test in the *E. coli* strain WP2 pKM101 in the absence of metabolic activation was also negative, but an increase in mutation frequency was observed in WP2 pKM101 in the presence of metabolic activation and in WP2 uvrA pKM101 regardless of metabolic activation. Alatrofloxacin did not induce an increase in mutation frequency in any of the *Salmonella* strains in the presence or absence of metabolic activation; however, a small increase in mutation frequency was observed in the *E. coli* strains in the presence (but not the absence) of

metabolic activation. In Chinese hamster ovary cells, neither trovafloxacin nor alatrofloxacin induced forward mutation at the HGPRT locus in the presence or absence of metabolic activation. Chromosomal aberrations were not induced in cultured human lymphocytes in the presence or absence of metabolic activation by trovafloxacin. Data from a similar assay with alatrofloxacin showed a small increase in abnormal cells, but lack of a clear dose-response relationship made interpretation of these data difficult. *In vivo*, chromosomal aberrations were not induced in bone marrow cells harvested from mice dosed orally with up to 1000 mg/kg of trovafloxacin or intravenously with up to 100 mg/kg of alatrofloxacin.

Trovafloxacin (given orally) and alatrofloxacin (given intravenously) did not affect the fertility of male or female rats at oral and IV doses of 75 mg/kg/day and 50 mg/kg/day, respectively. However, oral doses of trovafloxacin at 200 mg/kg/day were associated with increased preimplantation loss in rats. Alatrofloxacin induced skeletal variations and malformations in the offspring of rats and rabbits when intravenous doses ≥ 20 mg/kg/day were administered during the period of organogenesis. An increase in skeletal variations was observed in rat fetuses after daily oral 75 mg/kg maternal doses of trovafloxacin were administered during organogenesis. Maternal doses of intravenous alatrofloxacin at 50 mg/kg/day and oral trovafloxacin at 75 mg/kg/day were fetotoxic in rats (increased perinatal mortality and decreased body weights of offspring). Fetotoxicity and fetal skeletal malformations have been associated with other quinolones. Oral doses of trovafloxacin ≥ 5 mg/kg were associated with an increased gestation time in rats and several dams at 75 mg/kg experienced uterine dystocia.

The toxicities observed with trovafloxacin and alatrofloxacin in animal models are generally similar to those that have been observed with other fluoroquinolones. These include phototoxicity, induction of arthropathy in juvenile dogs, convulsions and other CNS disturbances, and teratogenicity (fetal skeletal malformations). None of these toxicities appears to be worse for trovafloxacin than for other approved fluoroquinolones. The liver toxicity observed in dogs (necrotizing inflammation of perivenular hepatocytes) was successfully monitored using an increase in serum liver enzymes as a marker and appeared reversible upon discontinuation of the drug.

RECOMMENDATION: The pharmacologist does not object to the approval of this NDA. The nonclinical data for trovafloxacin and alatrofloxacin are comparable to other quinolones marketed for clinical use. The label contains appropriate cautions regarding potential quinolone-related toxicities such as CNS effects, juvenile arthropathy, tendon rupture, and phototoxicity. The sponsor will submit
a Phase IV commitment.

/S/

APPROVED THIS WAY
DATE: 11/11/94

Amy L. Ellis, Ph.D.
Pharmacologist, HFD-520

Orig. NDA

cc:

HFD-520

HFD-590

HFD-520/Pharm Team Ldr/Osterberg

HFD-590/Pharm Team Ldr/Hastings

~~HFD-520/Pharm Team Ldr/Hastings~~

HFD-590/MO Team Ldr/Leissa

HFD-590/MO/Coyne

HFD-520/Chem/Shetty

HFD-590/CSO/Fogarty

HFD-520/Micro/Altaie

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/S/
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